

CLAIMS

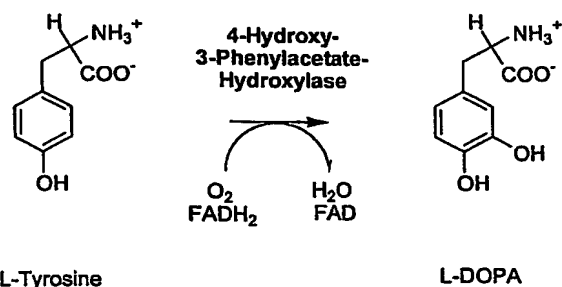
1. Process for the preparation of L-3,4-dihydroxyphenylalanine, wherein L-3,4,-
5 dihydroxyphenylalanine is produced in a fermentation medium by aerobic
fermentation of a recombinant microorganism having L-tyrosine-3-hydroxy-
mono-oxygenase activity and at least the metabolic pathways: glycolysis,
pentose phosphate pathway, aromatic amino acid pathway, or derivative
pathways thereof, wherein the process comprises
- 10 (i) a growth phase and a production phase, wherein L-3,4-dihydroxy-
phenylalanine is produced in the fermentation medium and
- (ii) a downstream processing phase,
characterized in that L-3,4-dihydroxy-phenylalanine is produced from a carbon
source and in that during at least part of the production phase and/or
downstream processing phase the pH is in the range of from 1 to 7.
- 15 2. Process according to claim 1, characterized in that in the downstream
processing phase the L-3,4-dihydroxy-phenylalanine produced is extracted
from the fermentation medium and reextracted into a reextraction mixture.
3. Process according to claim 1 or 2, characterized in that the pH of the
fermentation medium comprising L-3,4-dihydroxy-phenylalanine and/or the pH
20 of the reextraction mixture comprising L-3,4-dihydroxy-phenylalanine is in the
range of from 1 to 7 during the entire production phase of the fermentation
and/or during the entire downstream processing phase.
4. Process according to any of claims 1-3, characterized in that L-3,4,-
dihydroxyphenylalanine is recovered from the fermentation medium by
25 adsorption resins with a hydrophobic interactive surface and by subsequent
elution of the bound L-3,4,-dihydroxyphenylalanine from the resins with a
reextraction mixture.
5. Process according to any of claims 1-4, characterized in that L-3,4-
dihydroxyphenylalanine is extracted from the fermentation medium by *in situ*
30 product recovery.
6. Process according to claim 5, characterized in that *in situ* product recovery
comprises the steps of pumping the fermentation medium comprising L-3,4-
dihydroxyphenylalanine and the cells of the microorganism over a filter to
separate the cells from the fermentation medium, extracting L-3,4-
35 dihydroxyphenylalanine from the fermentation medium by reactive extraction

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and transferring L-3,4-dihydroxyphenylalanine into the reextraction mixture b
reextraction, and recycling of the cells and remaining fermentation medium to
the fermentation.

7. Process according to any of claims 1-6, characterized in that the recombinant
5 microorganism expresses, preferably overexpresses, a 4-
hydroxyphenylacetate 3-hydroxylase.
8. Process according to any of claims 1-7, characterized in that the recombinant
microorganism also expresses, preferably overexpresses, a gene encoding a
FADH₂-NAD-oxidoreductase.
- 10 9. Process according to any of claims 1-8, characterized in that the carbon
source is glucose.
10. Process according to any of claims 1-9, characterized in that the
microorganism is *Escherichia coli* W3110/pACYCtac *aroF^{FBR} tyrA*/pJF119Et
15 *aroF^{FBR} tyrA*.

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If the microorganism used in the invention does not naturally produce L-tyrosine-3-hydroxy-mono-oxygenase activity, the microorganism can be altered such as to produce this activity, for example by cloning and expression, preferably overexpression, of a gene encoding an enzyme with 4-hydroxyphenylacetate 3-hydroxylase activity (also known as 4-hydroxyphenylacetate 3-hydroxylase) into a suitable vector into the microorganism. Examples of genes encoding L-tyrosine-3-hydroxy-mono-oxygenases are: *PheA* encoding phenol hydroxylase from *Bacillus thermoleovorans*, *HpaA* encoding 4-hydroxyphenylacetate 3-hydroxylase from *Klebsiella pneumonia*, *hpaB* encoding 4-hydroxyphenylacetate 3-hydroxylase from *Escherichia coli*. Preferably, the gene encoding a 4-hydroxyphenylacetate 3-hydroxylase is the *hpaB* gene from *Escherichia coli* ATCC 11105.

Preferably, in the process according to the invention a microorganism is used that also expresses, and preferably overexpresses, a gene encoding a FADH₂-NAD-oxidoreductase. FADH₂-NAD-oxidoreductase enhances the activity of 4-hydroxyphenylacetate 3-hydroxylase (Xun *et al.* (200) Appl. Environ. Microbiol. vol 66: p 481-486). If the microorganism does not naturally have a gene encoding FADH₂-NAD-oxidoreductase or if the expression of the gene is too low, the microorganism can be altered such as to express this gene. For example, if the gene is not naturally present in the microorganism, the gene encoding FADH₂-NAD-oxidoreductase can be cloned in a suitable vector and introduced and subsequently expressed in the microorganism. Genes encoding FADH₂-NAD-oxidoreductase are for example described in Galan *et al.* (2000), J.Bacteriol. vol 182: p 627-636, for example the *hpaC* gene from *Escherichia coli* ATCC 11105B, the *fre* gene from *Escherichia coli*, the *hpaH* gene from *K.pneumoni*, the *hdaB* gene from *B. pickettii* etc.

Preferably, the genes encoding a 4-hydroxyphenylacetate 3-hydroxylase and a FADH₂-NAD-oxidoreductase are overexpressed in the microorganism. Overexpression can be achieved by methods known to the person

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